pISSN 2349-3283 | eISSN 2349-3291

Original Research Article

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20175587

Nucleated RBCs in umbilical cord blood as marker in cases of fetal asphyxia

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Received: 30 October 2017 Accepted: 25 November 2017

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ABSTRACT

Background: World health organization (WHO) has defined perinatal asphyxia as a failure to initiate and sustain breathing at birth. HIE is one of the most common complication in an asphyxiated neonate because of its serious long term neuromotor sequalae among the survivors. Nucleated red blood cells (NRBC) count in umbilical cord of newborns is been suggested as a sign of birth asphyxia. As the present markers are not accurate in diagnosis and assessing the severity of fetal asphyxia, this study was undertaken to find the values of NRBCs in normal and asphyxiated neonates and the correlation of NRBCs with birth asphyxia.

Methods: Eighty neonates with asphyxia along with eight healthy newborns were undertaken for two years study period. Maternal and neonatal information was recorded follow by clinical and laboratory evaluation. NRBC levels was determined per 100 white blood cells (WBC). After discharge, immediate follow-up of asphyxiated infants was performed. Neonates were divided into two groups, with favorable and unfavorable outcome based on discharge or death.

Results: We observed that NRBC count with more than 10 per 100 WBC/mm³, had sensitivity of 88.75% and specificity of 100% in predicting complications of asphyxia, while in NRBC count with more than 10, the sensitivity and specificity were 88.75% and of 100%, respectively.

Conclusions: We demonstrate that NRBC/100 WBC can be used as prognostic marker for neonatal asphyxia, which in combination with the severity of asphyxia could indicate high infant mortality, immediate outcome and complications of asphyxia

Keywords: Asphyxia, hypoxic ischemic encephalopathy, neonates, nucleated red blood cells

INTRODUCTION

Perinatal asphyxia word derived from the Greek word aspyxos, meaning born without an evident pulse, is one of the most important causes of fetal distress. Inspite of major advances in technology and knowledge of fetal and perinatal medicine, it is one of the significant causes of mortality and long-term morbidity. Asphyxia may have serious effects on many major and vital organs of neonates and can lead to respiratory distress syndrome, disseminated intravascular coagulation, necrosis of subcutaneous fat, myocardial ischemia, adrenal

hemorrhage, metabolic complications, acute tubular necrosis, neurological complications such as brain paralysis, convulsion and reduce in learning.¹⁻³

Asphyxia is one of the main causes of infant mortality and chronic neurologic disabilities in survived neonates. This condition occurs in 2-10% of deliveries.⁴⁻⁵ According to the reports of the World Health Organization (WHO) in developing countries, three percent of new-borns (6.3 million people) suffer from moderate or severe asphyxia, although 23% of them (840 000 people) die and approximately the same number of

subjects confront the complications of asphyxia.² Asphyxia is the cause of about one-fifth of neonatal deaths and the second leading cause of neonatal mortality after severe prematurity.⁶ Perinatal asphyxia can cause severe hypoxic-ischemic damages in the organs of neonates and cause severe long-term consequences or fatal complications.^{4,7} Thus, a reliable marker is required to predict the hospital stay and the prognosis of neonates with perinatal asphyxia.⁸

Nowadays, diagnosis of asphyxia is performed by several methods, including: apgar score, arterial blood gases, HIE signs, NRBCs, abnormal symptoms in electronic fetal monitoring during labor, as well as biochemical markers such as lactate, LDH, Peroxidant antioxidant balance and heat shock protein. 9-14 However there is no effective indicator for prediction of perinatal asphyxia although a combination of different methods and markers could help in the diagnosis of perinatal asphyxia. 15

It has recently been suggested that the increase in NRBC count in the umbilical cord blood of new-borns can be considered a sign of birth asphyxia. In healthy new-borns, NRBC count is reduced by half 12 hours after birth, and there are only 20-30 NRBCs/m³ 48 hours after birth. 17-20 On the other hand, tissue hypoxia results in increased levels of erythropoietin and erythrocytes. NRBC count reflects high production of erythropoietin; it means that erythropoietin stimulates fetal hematopoietic system, mainly in bone marrow, which increases the production of RBCs. 21-23

Increase in NRBC count is often due to prematurity, ABO or RH blood incompatibility, increase in hematopoiesis followed by chronic diseases, maternal diabetes, preeclampsia, fetal anemia, intrauterine infections, chorioamnionitis and acute or chronic asphyxia. ^{21,24-28} In the previous study, we performed preliminary diagnosis of perinatal asphyxia based on NRBC count. Boskabadi concluded that NRBC/100 WBC can be considered as simple markers for the evaluating of severity and primary consequences of perinatal asphyxia. ¹¹

Despite extensive effort in the pathophysiology of asphyxia, the severity of asphyxia and its long-term consequences is still remained as a major clinical concern. According to the high prevalence of asphyxia and its problems, in the current study we evaluated the maternal and neonatal risk factors for birth asphyxia, to study the clinical profile and also some specific investigations such as NRBCs and its correlation with severity of birth asphyxia.

METHODS

The current study was a prospective case control study with a study period of two years in Neonatal unit of Department of Pediatrics form August 2015 to July 2017 at Acharya Vinoba Bhave Rural Hospital, Sawangi,

Wardha that was conducted on 80 asphyxiated term and 80 normal neonates during 2015-2017.

Inclusion criteria

Neonates born at AVBRH who did not cried after birth and needed resuscitation at birth with Apgar score <7.

Exclusion criteria

Babies with any of the following criteria:

- New-borns whose parents didn't give consent
- Rh incompatibility
- Cyanotic congenital heart disease
- Hemolytic jaundice.

Babies delivered in our hospital and requiring resuscitation (basic and/or advanced) were included in the study after obtaining a written informed consent. Details of the mother were recorded, about the baby, resuscitation details and clinical assessments were recorded. These included asphyxia measures, NRBCs measure and outcome measures. Clearance was obtained from Institutional Ethical Committee.

Immediately after birth, 2 ml of the umbilical cord blood was collected in plain vial among both case and control group. Another 2-ml blood was collected in EDTA vial for routine haematological investigations to evaluate nucleated RBC's. Sample was assessed using Beckman Coulter machine and simultaneously a slide was prepared which was stained with Leishman stain, slide was then examined under oil immersion lens for NRBCs/100 WBC count. Cord blood pH was also noted to detect pH from serum, according to color of indicator.

As soon as baby was admitted to NICU, the details were entered in predesigned proforma which included detailed history regarding antenatal risk factors to perinatal asphyxia; age of mother, history of pregnancy induced hypertension, anaemia, bleeding, intrapartum factors like mode of delivery, h/o prolonged rupture of membrane, meconium stained liquor and malpresentation.

The short-term outcome at discharge was noted along with comorbidities if any with special mention of CNS status. Examination findings included vitals and detailed anthropometry with complete neurological and other system examination of the new-born.

Daily evaluation for detection of abnormal signs and symptoms was done. Grading of neonates according to Sarnat and Sarnat for HIE was done. Corelation of cord blood NRBC's/100 WBC with clinical condition at different stages of HIE in NICU was observed until discharge or death of neonate. The NRBC count of the case and control group is compared. The count of subjects belonging to different stages of HIE is then compared.

Statistical analysis was done by using descriptive and inferential statistics using chisquare test and Student's unpaired t-test and software used in the analysis were SPSS 17.0 version and GraphPad Prism 6.0 version. Pearson correlation coefficient was performed using SPSS 11.5 software. Receiver-operating characteristic (ROC) curves were also constructed allowing the calculation of positive and negative predictive values. A p $<\!0.05$ is considered as level of significance. The results were tested at 5% level of significance.

RESULTS

The mean age of mothers was 24.36 years in cases and 24.65 in controls, which ranged from 20 years to 29 years. Most of the newborns with PA were born to mothers from lower middle and upper lower class (Class 3 + Class 4). 50% neonates were born to primiparous mothers and birth asphyxia was most common in babies born to primipara mothers.

Table 1: Distribution according to neonatal factors.

Neonatal factors	Case group		Control group		4 malma	
Neomatai factors	Mean	SD	Mean	SD	t-value	p-value
Birth Weight(kg)	2.21	0.22	2.53	0.43	4.04	0.0001, S
	Case group	%	Control group	%	χ²-value	p-value
Gestation						
Term	58	72.5	55	68.75	0.27	0.60, NS
Preterm	22	27.5	25	32.25	0.27	
Presentation						
Vertex	73	91.25	80	100		0.025, S
Breech	6	7.5	0	0	7.32	
Face	1	1.25	0	0		
Delivery type						
LSCS	42	52.5	48	60	0.01	0.33, NS
NVD	38	47.5	32	40	0.91	
MSAF	9	11.25	17	21.25	3.72	0.05, NS
Distress	10	12.5	0	0	10.67	0.0001, S

Table 2: Comparison of the combination of indicators that predict the risk of birth asphyxia.

Birth asphyxia	HIE Staging	Mean APGAR 1 minute	Mean APGAR 5 minutes	Mean cord blood pH	Mean cord blood NRBCs
Without HIE	No	9.04±1.11	9.79 ± 0.48	7.43 ± 0.08	1.31±4.66
With HIE	1	5.86±1.37	8.28±0.92	7.24 ± 0.18	29.50±23.41
	2	3.94±2.07	6.64±2.37	6.92±0.24	107.17±120.65
	3	3.31 ± 2.12	5.81±1.22	6.21±0.27	253.62±116.03
F-value		112.25	76.02	26.60	107.05
p-value		0.0001, S	0.0001, S	0.0001, S	0.0001, S

Table 3: Categorization of asphyxiated new-borns on the basis of HIE staging.

Outcome at discharge	No HIE	HIE I	HIE II	HIE III	Total	
CNS Normal	9	38	5	0	52	
CNS Abnormal	0	0	2	1	3	
Death	0	1	9	15	25	
Total	9	39	16	16	80	

Anemia was widely prevalent in the mothers of neonates requiring resuscitation. The maternal risk factors for newborns requiring resuscitation were PIH (23.7%), oligohydramnios (20%), polyhydramnios (11.25%), multiple gestation (3.75%), PROM (2.5%), GDM (2.5%), chorioamnionitis (2.5%), bleeding PV (2.5%) and

eclampsia (1.25%). One third of neonates requiring resuscitation were born to unbooked mothers.

In the neonates requiring resuscitation, out of 80 babies in cases 44 (55%) and 36 (45%) were male and female respectively. The male to female ratio was 1.22:1 with marginal male preponderance. Mean birth weight found

was 2.21 kg in cases and 2.53 kg in controls. The mean birth weight of babies who died was 1.95 kg while of those who got discharged was 2.37 kg. This difference was statistically significant. Birth weight was found to be lower in babies with more severe degrees of HIE. 72.5% of asphyxiated babies were full term, 52.25% asphyxiated babies born out of caesarean section and 91.25% of

asphyxiated babies born out of vertex delivery with LSCS:NVD ratio being 1.1:1. PA is more commoner in term babies. The fetal factors associated with resuscitation of new-borns were prematurity (27.5%), IUGR (23.75%), fetal distress (12.5%), MAS (11.25%) and malpresentations (5%).

Table 4: Categorisation of new-borns as discharge or death on the basis of birth weight.

Birth weight (kg)	Discharge	Death	Total	χ2-value
Upto 1.5 kg	2 (25%)	6 (75%)	8 (10%)	
1.51 to 2 kg	8 (47.05%)	9 (52.94%)	17 (21.25%)	11 12
2.01-2.5 kg	30 (78.95%)	8 (21.05%)	38 (47.50%)	11.12 $p = 0.011, S$
>2.5 kg	15 (88.23%)	2 (11.76%)	17 (21.25%)	p = 0.011, S
Total	55 (68.75%)	25 (31.25%)	80 (100%)	

Table 5: Comparison of the final outcomes (discharge/death) for neonates at different NRBC count levels.

Outcome	N	Mean	Std. Deviation	Std. Error Mean	t-value
Discharge	55	16.1	37.52	5.10	7.18
Death	25	70.4	97.13	19.4	p=0.0001,S

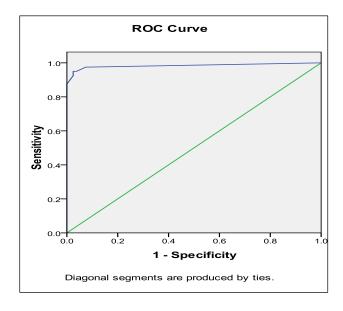


Figure 1: ROC curve for comparison of sensitivity and specificity of NRBCs count in asphyxiated neonates.

72.5% asphyxiated babies had a duration of stay of 3-14 days with mean duration of stay was 9.06 days. 87.5% of neonates in this study were revived on bag and mask ventilation alone, followed by endotracheal intubation (23.75%), ET suction (13.75%), chest compressions (12.5%) and medications (8.75%).

The mean APGAR at 1 minute was 4.17 in cases and 9.37 in controls and at 5 minutes was 6.68 in cases and 9.92 in controls with p-value being significant. Mean pH

was 7.05 in cases and 7.45 in controls with p-value being significant. CRP came to be positive in 35% of asphyxiated newborns. Worsening umbilical cord pH correlated with increasing severity of HIE and also with mortality.

47.5% of asphyxiated babies belonged to stage I, followed by 21.25 in stage II and 20% in stage III HIE. NRBCs were found to be 76.25% between 11-100 / 100 WBC counts. As the pH increased NRBCs decreased. Mean number of NRBCs was $68.62{\pm}10.78$ in the asphyxiated newborns. On correlating NRBCs with severity of acidosis, it was significant.

NRBCs count correlate well with APGAR score at 1 minute and 5 minutes. As the APGAR score increased, mean NRBCs counts decreased. As the HIE stage increased, mean cord pH and mean APGAR decreased but mean cord blood NRBCs increased and p-value was significant. In ROC, area under the curve was 0.98 with significant p-value.

NRBCs with cutoff ≥ 10 has sensitivity of 88.75%, specificity of 100%, positive predictive value of 100% and negative predictive value of 89.89%. Pearson's correlation coefficient depicts negative co-relation between cord blood pH and NRBCs i.e. as the cord blood pH increases NRBCs count decreases and has significant p-value.

67.5% and 42.5% of asphyxiated babies required antibiotics and anticonvulsants respectively. 48.75% asphyxiated babies belong to HIE stage I, 20% to stage II

and 20% to stage III. 11.25 % of asphyxiated babies do not land up in HIE. Increasing vasopressor use was associated with severity of HIE. 55 asphyxiated newborns who were discharged had a mean NRBCs count of 16.1 and 70.4 in 25 new-borns who died, and p-value was significant.

DISCUSSION

We illustrated that NRBC count in combination with HIE grade can prognostic the risk of complications of asphyxia. It has been documented that most of acute and chronic condition could increase NRBC count through boosting the erythropoietic activity. Previous studies have been suggested NRBC as hematopoietic marker in neonates and also its relationship with intrauterine hypoxia. Oracle Thus, in hypoxic conditions such as in perinatal asphyxia, a compensatory response is created as increased erythropoiesis, and then NRBCs release in the infant's blood circulation. Moreover, it has been suggested that increased NRBC count is not only the marker of perinatal asphyxia, but also predicts the risk of consequences of neurological development.

Although the exact mechanism is unclear, it may be relevant to increase the umbilical cord blood erythropoietin levels in 1 to 4 hours after acute asphyxia. Increased secretion of erythropoietin results in increased release of NRBCs from the bone marrow due to increased mitotic divisions of normoblasts.^{25,26} Present data showed that in asphyxiated neonates with unfavourable outcomes, NRBC count/WBC had higher values but pH had lower values. Ghosh et al. found a negative relationship between the NRBCs level, Apgar score and pH of umbilical artery.15 Although, Rai et al. showed that NRBCs in asphyxiated neonates with unfavourable outcomes was higher.8 Also, high levels of NRBC had a relationship with severe acidosis, low Apgar scores, low platelet count and poor short-term outcomes.¹¹ Another study by Niroomanesh and colleagues showed that a reverse relationship between NRBC count in umbilical cord blood and fifth-minute Apgar score and umbilical cord blood pH.37

Similarly, Hanlon-Lundberg et al revealed that increased in NRBC count was related by a progressive increasing in umbilical acidosis.³⁸ In line with this data, Ferns and coworkers showed that NRBC count in asphyxiated neonates was markedly higher. These observations provide proofs of concept of NRBC count at birth as a helpful marker in predicting the severity of asphyxia and also prognosis of the neonate in a short-term period.³⁹ Consistent with previous findings, unfavorable outcomes were increased by increasing HIE grades, which is in line with our previous data.^{4,40} Several other studies have also showed the association of HIE grade with neurological development in neonates. Thus, early detection and treatment of neurological development disorders are essential.⁴¹⁻⁴³

In the present study, HIE was also emerged as one of the major causes of morbidity and mortality in asphyxiated neonates. In addition to the value of HIE, several studies, including Minior et al. showed that newborns with low-birth-weight had high NRBC count, needed mechanical ventilation or other blood pressure regulators. ⁴⁴ Present data showed that NRBC, HIE grades, first-hour pH and the need for mechanical ventilation are of important indicators of the prognosis in asphyxiated neonates.

Additionally, we observed that NRBC count with more than 10, had sensitivity of 88.75% and specificity of 100% in predicting the complications of asphyxia, which is in somehow in line with our previous data. Similarly Khurana et al. in 2014 suggested that NRBC count with more than 10/100 WBC had sensitivity of 86% and specificity of 100% for predicting the severity of perinatal asphyxia.

CONCLUSION

In conclusion, present results showed that the NRBCs count in cord blood at birth can be considered as a good marker of PA. A simple, cheap, rapid and non-invasive test of cord blood NRBCs count provides valuable information about the well-being of the new-born at birth and it correlates well with APGAR score and cord blood pH. The NRBCs count can be used as a simple tool to predict the severity and immediate outcome of birth asphyxia with sensitivity of 88.75%, specificity of 100%, PPV of 100% and NPV of 89.89%.

ACKNOWLEDGEMENTS

Authors would like to thank and appreciate officials and everyone who helped them in performing this project.

Funding: No funding sources Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee Ref No. IEC/2015-16/1495

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Cite this article as: Shrivastava A, Vagha J, Borkar R. Nucleated RBCs in umbilical cord blood as marker in cases of fetal asphyxia. Int J Contemp Pediatr 2018;5:203-8.